EPIGENTEK
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EpiQuik™ Plant ChIP Kit

Base Catalog # P-2014

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik*™ Plant ChIP Kit is suitable for combining the specificity of immunoprecipitation with qualitative and quantitative PCR, MS-PCR, DNA sequencing, and southern blot, as well as DNA microarray.

Like using other ChIP kits, if you use the *EpiQuik*™ Plant ChIP Kit, choice of a good antibody (i.e. IP proved) is required for precipitating the fixed protein/DNA complexes.

The kit size is designed for 24 or 48 ChIP reactions, not for 24 or 48 samples. The standard protocol of the kit allows for performing 8 reactions with one sample. For testing more samples, the amount of each sample should be reduced. The amount of each component of the kit or reagents used for chromatin preparation should be also proportionally reduced.

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KIT CONTENTS

Important Information: The amount of components supplied in this kit is designed for reaction count, not sample count, such as negative IgG controls and input DNA. Thus, experiments with samples to be paired with both IgG and input may require additional columns or components to be purchased separately. Please calculate the necessary volumes based on the below kit contents and protocol prior to starting the experiment.

Components	24 reactions P-2014-24	48 reactions P-2014-48
CP1 (Wash Buffer)	28 ml	2 x 28 ml
CP2 (Antibody Buffer)	15 ml	30 ml
CP3C (5X Lysis Buffer I)	12 ml	24 ml
CP3D (Lysis Buffer II)	3 ml	6 ml
CP3E (Lysis Buffer III)	2 ml	4 ml
CP3F (Lysis Buffer IV)	1.5 ml	5 ml
CP4 (ChIP Dilution Buffer)	2 ml	6 ml
CP5 (DNA Release Buffer)	2 ml	2 x 2 ml
CP6 (Reverse Buffer)	2 ml	2 x 2 ml
CP7 (Binding Buffer)	5 ml	8 ml
CP8 (Elution Buffer)	0.6 ml	1.2 ml
Protease Inhibitor Cocktail (100X)*	25 µl	50 µl
Non-immune IgG (1 mg/ml)*	10 µl	10 µl
Anti-Dimethyl H3-K9 (1 mg/ml)*	5 µl	8 µl
Proteinase K (10 mg/ml)*	25 µl	50 µl
8-Well Assay Strips (with frame)	3	6
8-Well Strip Caps	3	6
F-Spin Column	30	50
F-Collection Tube	30	50
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** Spin the solution down to the bottom before use.*

SHIPPING & STORAGE

The kit is shipped in two parts: one part at ambient room temperature, and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store the following components at 4°C: **Protease Inhibitor Cocktail**, **Non-immune IgG**, **Proteinase K**, **Anti-Dimethyl H3-K9** and **8-Well Assay Strips**. (2) Store **all other components** at room temperature.

The kit is stable for up to 6 months from the shipment date, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Variable temperature waterbath
- ☐ Vortex mixer
- ☐ Desktop centrifuge (up to 14,000 rpm)
- ☐ Dounce homogenizer
- ☐ Sonicator
- ☐ Orbital shaker
- ☐ Miracloth
- ☐ Nylon mesh
- ☐ desiccator attached to a vacuum pump
- ☐ Pipettes and pipette tips
- ☐ 1.5 ml microcentrifuge tubes
- ☐ 15 ml conical tube
- ☐ 50 ml Falcon tube
- ☐ 100 mm plate
- ☐ Formaldehyde
- ☐ Glycine
- ☐ β-mercaptoethanol
- ☐ Antibody of interest
- ☐ TE buffer (pH 8.0)
- ☐ Ethanol (96-100%)
- ☐ deionized water

GENERAL PRODUCT INFORMATION

Safety: Suitable lab coat and disposable gloves are required when working with the kit.

Quality Control: Epigentek guarantees the performance of all products in the manner described in our product instructions.

Product Updates: Epigentek reserves the right to change or modify any product to enhance its performance and design.

Usage Limitation: The *EpiQuik*™ Plant ChIP Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The *EpiQuik*™ ChIP kits and methods of use contain proprietary technologies by Epigentek. *EpiQuik*™ is a trademark of Epigentek, Inc.

A BRIEF OVERVIEW

Protein-DNA interaction play a critical role for cellular functions such as signal transduction, gene transcription, chromosome segregation, DNA replication and recombination, and epigenetic silencing. In plants, interactions between the DNA-binding proteins and cognate promoter sequences are primary determinants in establishing spatial and temporal expression patterns of genes that effect homeostasis, development, and adaptation.

Chromatin Immunoprecipitation (ChIP) offers an advantageous tool for identifying direct genome-wide associations between specific regulatory proteins and their target genes. Unlike other methods such as EMASA, DNA microarrays, and report gene assays, which analyze direct interactions between protein and DNA *in vitro*, ChIP can detect that a specific protein binds to the specific sequences of a gene in living cells.

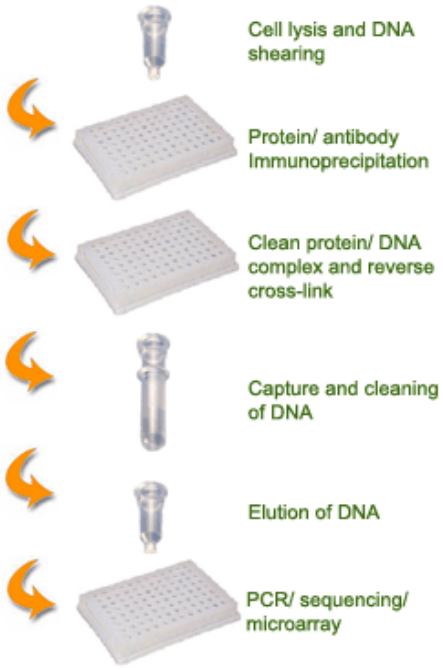
There are several methods used for chromatin immunoprecipitation, however, most of these methods available so far are considerably time consuming, labor intensive, or have low throughput. The *EpiQuik*™ ChIP kits use a proprietary and unique procedure and composition to investigate protein-DNA interaction *in vivo* efficiently. The *EpiQuik*™ ChIP kit series have the following features:

- The fastest procedure currently available, which can be finished within 6 hours.
- Strip microplate format makes the assay flexible: manual or high throughput.
- Columns for DNA purification are included: save time and reduce labor.
- Compatible with all DNA amplification-based approaches.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The *EpiQuik*™ Plant ChIP Kit contains all reagents required for carrying out a successful chromatin immunoprecipitation from plant cells. Particularly, this kit includes a ChIP-grade dimethyl-histone H3-K9 antibody and a negative control Non-immune IgG. Chromatin from the cells is extracted, sheared, and added into the microwell immobilized with the antibody. DNA is released from the antibody-captured protein-DNA complex, reversed, and purified through the specifically designed Fast-Spin Column. Eluted DNA can be used for various down-stream applications.

Schematic Procedure for Using the *EpiQuik*™ Plant ChIP Kit



PROTOCOL

Note: Always cap spin columns before placing them in the microcentrifuge.

Before starting, perform the following:

1. Prepare the following required solutions (not included): 90% Ethanol; 70% Ethanol; 37% Formaldehyde; 2M Glycine Solution; 14.3M βeta-mercaptoethanol (BME); 1X TE Buffer (pH 8.0).
2. Ensure that all buffers are in clear solution. Shake or vortex if these buffers precipitate.

Antibody Binding to the Assay Plate

1. Determine the number of strip wells required. Leave these strips in the plate frame (remaining unused strips can be placed back in the bag. Seal the bag tightly and store at 4°C). Wash strip wells once with 150 µl of CP1.
2. Add 100 µl of CP2 to each well and then add the antibodies: 1 µl of Non-immune IgG as the negative control, 1 µl of Anti-Dimethyl H3-K9 as the positive control, and 2-3 µg of an *antibody of interest*.
3. Cover the strip wells with Parafilm M and incubate at room temperature for 60-90 minutes. After incubation, remove the incubated antibody solution and wash the strip wells three times with 150 µl of CP2 by pipetting in and out. (During incubation time, the cell extracts can be prepared as described in the next steps.)

Tissue Collection and *In Vivo* Cross-Link

1. Harvest 0.8-1g of plant tissue (flowers, leaves, or young seedlings) after growth on soil or *in vitro* in a 50 ml Falcon tube.

2. Rinse tissue gently with 20 ml of deionized water two times. Remove as much water as possible from the tissue and add 20 ml of *1.0% formaldehyde*.
3. Stuff the top of the 50 ml conical tube (containing the *formaldehyde* soaked tissue) with nylon mesh to keep the tissue immersed during vacuum infiltration (and aid later rinse steps). Also poke needle-sized holes in the cap of the conical tube and screw on the cap.
4. Vacuum infiltrate the tissue for 10 minutes in a desiccator attached to a vacuum pump. The formaldehyde solution should boil.

Tissue Lysis and DNA Shearing

1. Quench cross-linking by adding 1.25 ml of *2M Glycine solution* (final concentration 0.125M) and continue vacuum infiltration for an additional 5 minutes.
2. Remove the *formaldehyde* and rinse the tissue two times with 20 ml of deionized water. After the rinses, remove as much water as possible (at this stage the cross-linked tissue can either be frozen in liquid nitrogen and stored at –80°C, or used directly for chromatin extraction).
3. Dilute **CP3C** with distilled water at a 1:5 ratio (**1X CP3C**). Add 3.5 µl of *BME* to each 10 ml of **1X CP3C**. Grind the tissue in liquid nitrogen to a fine powder. Add the powder to 20 ml of cold **1X CP3C** in a 50 ml conical tube, then vortex, and place on ice.
4. Filter solution through two layers of Miracloth into a 50 ml tube and centrifuge the filtered solution at 4000 rpm (1900X g) for 20 minutes.
5. Add 1 µl of *BME* into each 1 ml of **CP3D**. Remove supernatant and re-suspend pellet in 1 ml of **CP3D** containing *BME*. Transfer the re-suspended pellet to a 1.5 ml vial and centrifuge at 12,000 rpm for 10 minutes at 4°C to pellet nuclei (white pellet should be seen at this stage).
6. Add 1 µl of *BME* into each 1 ml of **CP3E**. Remove supernatant and re-suspend pellet in 300 µl of **CP3E** containing *BME*.
7. Add 300 µl of **CP3E** containing *BME* into a new 1.5 ml vial. Layer the resuspended pellet from step 6 on top of this 300 µl cushion and centri fuge at 14,000 rpm for 45 minutes at 4°C.
8. Remove supernatant and re-suspend chromatin pellet in 500 µl of **CP3F** containing **Protease Inhibitor Cocktail (PIC)** (ex: 10 µl of **PIC** to each 1 ml of **CP3F**). Shear DNA by sonication. For example, sonicate chromatin solution on ice five times, 15 seconds each at 40% duty cycle; power setting 2 on a Branson sonifier (Kranz lab). Place the sample on ice for 1 minute between each sonication treatment. (*The conditions of cross-linked DNA shearing can be optimized based on cells and sonicator equipment. If desired, remove 5 µl of sonicated cell lysate for agarose gel analysis. The length of sheared DNA should be between 200-1000 bp.*)
9. Pellet cell debris by centrifuging at 14,000 rpm for 10 minutes at 4°C.

Protein/DNA Immunoprecipitation

1. Transfer clear supernatant to a new 1.5 ml vial. (supernatant can be stored at –80°C at this step.) Dilute the required volume of supernatant with **CP4** at a 1:1 ratio (ex: add 100 µl of **CP4** to 100 µl of supernatant).
2. Remove 5 µl of the *diluted supernatant* to a 0.5 ml vial. Label the vial as "input DNA" and then place on ice.
3. Transfer 100 µl of the *diluted supernatant* to each antibody-bound strip well. Cover the strip wells with Parafilm M and incubate at room temperature (22-25°C) for 60-90 minutes on an orbital shaker (50-100 rpm).

4. Remove supernatant. Wash the wells six times with 150 µl of **CP1**. Allow 2 minutes on a orbital shaker (100 rpm) for each wash. Wash the wells once (for 2 minutes) with 150 µl of *1X TE Buffer*.

Cross-Linked DNA Reversal/DNA Purification

1. Add 1 µl of **Proteinase K** to each 40 µl of **CP5** and mix. Add 40 µl of **CP5** containing **Proteinase K** to the samples (including the "input DNA" vial). Cover the sample wells with strip caps and incubate at 65°C in a waterbath for 15 minutes.
2. Add 40 µl of **CP6** to the samples; mix, and re-cover the wells with strip caps and incubate at 65°C in a waterbath for 90 minutes. Also add 40 µl of **CP6** to the vial containing *supernatant*, labeled as "input DNA." Mix and incubate at 65°C for 90 minutes.
3. Place a spin column into a 2 ml collection tube. Add 150 µl of **CP7** to the samples and transfer mixed solution to the column. Centrifuge at 12,000 rpm for 20 seconds.
4. Add 200 µl of *70% ethanol* to the column, centrifuge at 12,000 rpm for 15 seconds. Remove the column from the collection tube and discard the flowthrough.
5. Replace column to the collection tube. Add 200 µl of *90% ethanol* to the column and centrifuge at 12,000 rpm for 20 seconds.
6. Remove the column and discard the flowthrough. Replace the column to the collection tube and wash the column again with 200 µl of *90% ethanol* at 12,000 rpm for 35 seconds.
7. Place the column in a new 1.5 ml vial. Add 10-20 µl of **CP8** directly to the filter in the column and centrifuge at 12,000 rpm for 20 seconds to elute purified DNA.

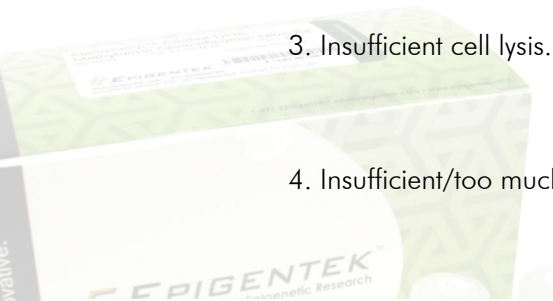
DNA is now ready for use or storage at –20°C.

Note: *For PCR positive control, the primers for At4g03770 or At4g03800 could be used, which represent retrotransposons located within the heterochromatic knob on chromosome 4 of Arabidopsis thaliana and are associated with di-methylated H3-K9. For conventional PCR, the number of PCR cycles may need to be optimized for better PCR results. In general, the amplification difference between "normal IgG control" and "positive control" may vary from 3 to 8 cycles, depending on lysate condition (fresh or frozen).*

TROUBLESHOOTING

Little or No PCR Products

1. Insufficient tissues.	Increase tissue amount (ex: >10 mg of tissue/per reaction).
2. Insufficient or too much cross-linking.	Check if the appropriate cross-link step is carried out according to the protocol.
3. Insufficient cell lysis.	Follow the guidelines in the protocol. Check the cell lysis by observing a 5 µl portion of the tissue lysate under the microscope.
4. Insufficient/too much sonication.	Follow the protocol instruction for obtaining the appropriate sized DNA. Keep the sample on ice during the sonication.



5. Incorrect temperature or insufficient time for DNA release and reversal of cross-linking.	Follow the guidelines in the protocol for appropriate temperature and time.
6. Incorrect PCR conditions.	Check if all PCR components are added. Increase the amount of DNA added to PCR reaction. Increase the number of cycles for PCR reaction.
7. Incorrect or bad primers.	Ensure the designed primers are specific to the target sequence.
8. The column is not washed with 90% ethanol.	Ensure that wash solution is 90% ethanol.
9. DNA is not completely passed through filter	Increase centrifuge time to 1 minute at steps 3-7 of "Cross-Linked DNA Reversal/DNA Purification."

Little or No Amplification Difference Between the Sample and the Negative Control

1. Insufficient wash at each wash step.	Follow the protocol for appropriate wash.
2. Antibody is added into the well for the negative control by mistake.	Ensure antibody is added into the correct well.
3. Too many PCR cycles.	If using conventional PCR, decrease the cycles to appropriate cycle number. Differences between quantities of starting DNA can be measured generally within the linear PCR amplification phase.
4. Little or no enrichment of the target protein in target promoters.	N/A.

RELATED PRODUCTS

P-2002	*EpiQuik*™ Chromatin Immunoprecipitation (ChIP) Kit
P-2006	*EpiQuik*™ Methyl-Histone H3-K9 ChIP Kit
P-2007	*EpiQuik*™ Methyl-Histone H3-K4 ChIP Kit
P-2010	*EpiQuik*™ Acetyl-Histone H3 ChIP Kit
P-2011	*EpiQuik*™ Acetyl-Histone H4 ChIP Kit